Ann. Occup. Hyg., Vol. 53, No. 7, pp. 759–769, 2009
Published by Oxford University Press
on behalf of the British Occupational Hygiene Society
doi:10.1093/annhyg/mep051

N-Acetyl-*S*-(*n*-Propyl)-L-Cysteine in Urine from Workers Exposed to 1-Bromopropane in Foam Cushion Spray Adhesives

KEVIN W. HANLEY[1]*, MARTIN R. PETERSEN[1], KENNETH L. CHEEVER[2] and LIAN LUO[3]

[1]Division of Surveillance, Hazard Evaluations and Field Studies, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Cincinnati, Ohio; [2]Division of Applied Research and Technology, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Cincinnati, Ohio; [3]Constella Group, Inc., Cincinnati, Ohio

Received 14 November 2008; in final form 12 June 2009; published online 25 August 2009

1-Bromopropane (1-BP) has been marketed as an alternative for ozone depleting and other solvents; it is used in aerosol products, adhesives, metal, precision, and electronics cleaning solvents. Mechanisms of toxicity of 1-BP are not fully understood, but it may be a neurological and reproductive toxicant. Sparse exposure information prompted this study using 1-BP air sampling and urinary metabolites. Mercapturic acid conjugates are excreted in urine from 1-BP metabolism involving debromination. Research objectives were to evaluate the utility of urinary *N*-acetyl-*S*-(*n*-propyl)-L-cysteine (AcPrCys) for assessing exposure to 1-BP and compare it to urinary bromide [$Br^{(-)}$] previously reported for these workers. Forty-eight-hour urine specimens were obtained from 30 workers at two factories where 1-BP spray adhesives were used to construct polyurethane foam seat cushions. Urine specimens were also obtained from 21 unexposed control subjects. All the workers' urine was collected into composite samples representing three time intervals: at work, after work but before bedtime, and upon awakening. Time-weighted average (TWA) geometric mean breathing zone concentrations were 92.4 and 10.5 p.p.m. for spraying and non-spraying jobs, respectively. Urinary AcPrCys showed the same trend as TWA exposures to 1-BP: higher levels were observed for sprayers. Associations of AcPrCys concentrations, adjusted for creatinine, with 1-BP TWA exposure were statistically significant for both sprayers ($P < 0.05$) and non-sprayers ($P < 0.01$). Spearman correlation coefficients for AcPrCys and $Br^{(-)}$ analyses determined from the same urine specimens were highly correlated ($P < 0.0001$). This study confirms that urinary AcPrCys is an important 1-BP metabolite and an effective biomarker for highly exposed foam cushion workers.

Keywords: bromide; 1-bromopropane; CAS No. 106-94-5; foam cushion; *N*-acetyl-*S*-(*n*-propyl)-L-cysteine; spray adhesive; urine

INTRODUCTION

An international agreement restricts the use of ozone-depleting substances including some compounds which were widely used throughout the industry: 1,1,1-trichloroethane and chlorofluorocarbons (freons®). An industry consortium petitioned the Environmental Protection Agency (EPA) to accept 1-bromopropane (1-BP) as an alternative for ozone-depleting solvents for aerosols; adhesives; metals, electronics, and precision cleaning (EPA, 1999; EPA, 2000). Products containing potential carcinogens trichloroethylene, perchloroethylene, and methylene chloride are also candidates for substitute solvents. Future applications for 1-BP may include paint, printing ink, asphalt cement, and textile cleaning solvents (Dead Sea Bromine, 1999; Petroferm Inc., 2000; EnviroTech International, Inc., 2008). EPA published a final rule to accept 1-BP in solvent cleaning and a proposed rule to reject it in adhesives and aerosols (EPA, 2007). The quest for alternative solvents could expand 1-BP markets potentially exposing many workers.

Currently, occupational exposure limits for 1-BP are not available from the National Institute for

*Author to whom correspondence should be addressed.
Tel: 513-841-4113; fax: 513-841-4486;
e-mail: KHanley@cdc.gov

Occupational Safety and Health (NIOSH, 1992) nor the Occupational Safety and Health Administration (OSHA, 2009). Manufacturers' exposure guidelines are inconsistent, ranging from 10 to 100 parts per million (p.p.m.) (Great Lakes Chemicals, 2005; EnviroTech International, Inc., 2006). The American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Value® (TLV®) for 1-BP is a 10 p.p.m., 8-h time-weighted average (TWA) based on suspected embryonic/fetal, neurological, and hepatic toxicity (ACGIH, 2009).

Based on concern regarding toxicity, NIOSH requested that the National Toxicology Program (NTP) evaluate 1-BP (NTP, 2004). The absence of exposure information resulted in an interagency agreement between NTP and NIOSH to conduct an occupational exposure study in multiple industries. In this study, 1-BP exposures were measured for most jobs at two factories using spray adhesives to manufacture foam furniture cushions. The underlying goal of this study was to evaluate a new high performance liquid chromatography (HPLC) electrospray ionization mass spectrometry (ESI-MS) method for characterizing mercapturic acid conjugates possible from 1-BP metabolism. Specific objectives were to measure urinary *N*-acetyl-*S*-(*n*-propyl)-L-cysteine (AcPrCys) levels and determine if a relationship of AcPrCys existed with workers' 1-BP exposures and their urinary bromide [$Br^{(-)}$] levels previously published for these workers (Hanley *et al.*, 2006).

BACKGROUND

A number of animal toxicity studies have implicated 1-BP as a reproductive or neurological toxicant (Ichihara *et al.*, 2000a,b; Takeuchi *et al.*, 2001; Yu *et al.*, 2001; Wang *et al.*, 2003; Yamada *et al.*, 2003; NTP, 2004; Fueta *et al.*, 2007; Lee *et al.*, 2007; Ueno *et al.*, 2007; Yoshida *et al.*, 2007). Ichihara (2005) concluded that, in rats, 1-BP was a neurotoxicant which produced prolonged distal latencies; decreased nerve conduction velocities; cerebellum degeneration; peripheral myelin sheath deterioration; and muscle weakness.

Health surveys were conducted by Ichihara *et al.* (2004a,b) at a factory after production changed from 2-bromopropane to 1-BP synthesis. Significant changes in motor nerve conduction velocities were not observed but decreased vibration sensation in toes, longer distal latencies, and neurobehavioral deficits were noted for exposed workers. Ichihara proposed that 1-BP may produce adverse peripheral sensory, motor, or central nervous system effects in humans.

Majersik *et al.* (2007) reported on a case study of six foam cushion gluers with neurological dysfunction after using 1-BP-based adhesives. The mean TWA 1-BP exposure was determined to be 108 p.p.m. (range: 92–127 p.p.m.) after additional ventilation was used. Symptoms included lower extremity pain, numbness, weakness, labored walking, headache, and nausea. Serum $Br^{(-)}$ levels were 44–170 mg dl^{-1} (reference: 0–40 mg dl^{-1}) with hyperchloremia measured at 107–139 mmol l^{-1} (reference: 98–107 mmol l^{-1}). A 2-year follow-up revealed that the two workers most severely affected had little improvement and three patients suffered chronic neuropathic pain. The authors concluded that 1-BP exposure may produce neurotoxicity in humans, principally spastic paraparesis with distal sensory loss.

Raymond and Ford (2007) described another case study involving four workers exposed to 1-BP adhesives following their hospitalization. Mean TWA 1-BP exposure, measured 9 months later, was slightly over 100 p.p.m. Primary symptoms were consistent with previous case studies: numbness, tingling, weakness, and reduced sensation in lower legs with unsteady gait. Elevated $Br^{(-)}$ in serum was determined to range from 3 to 12.5 mEq l^{-1} (reference < 0.6 mEq l^{-1}). In the two workers not lost to follow-up, symptoms persisted after 8 years, albeit less severely. The authors concluded that severe neurological disease occurred in these workers but concurrent elevated arsenic levels, perhaps acting synergistically with $Br^{(-)}$, prevented a definitive determination.

Metabolism of 1-BP is relatively complex and occurs by several pathways shown in Fig. 1, which includes exhalation of unaltered 1-BP; debromination; oxidation by CYP2E1; and S-conjugation with glutathione (Barnsley *et al.*, 1966; Jones and Walsh, 1979, 1980; Garner *et al.*, 2006). An intermediate metabolite, *S*-*n*-propyl-glutathione, is cleaved to *S*-*n*-propyl-L-cysteine which is metabolized producing several mercapturic acid conjugates excreted in urine. Bromide ions are eliminated mainly through the kidneys in urine (Ryan and Baumann, 1999). Urinary $Br^{(-)}$ elevated substantially above normal (>10 mg l^{-1}) represents accumulated exposure over the previous week or two, as $Br^{(-)}$ is slowly excreted (Jones and Walsh, 1979). Even though $Br^{(-)}$ analysis is well established, it may not be ideal for evaluating 1-BP exposure because background levels may be influenced by diet, medications, and exposure to other brominated compounds. Hence, mercapturic acid conjugates which have been used as biomarkers of exposure to halogenated compounds (Van Welie *et al.*, 1992; De Rooij *et al.*, 1998) may prove more specific for occupational exposure to 1-BP than urinary $Br^{(-)}$.

METHODS

This is a follow-up manuscript regarding two factories manufacturing furniture cushions (Hanley

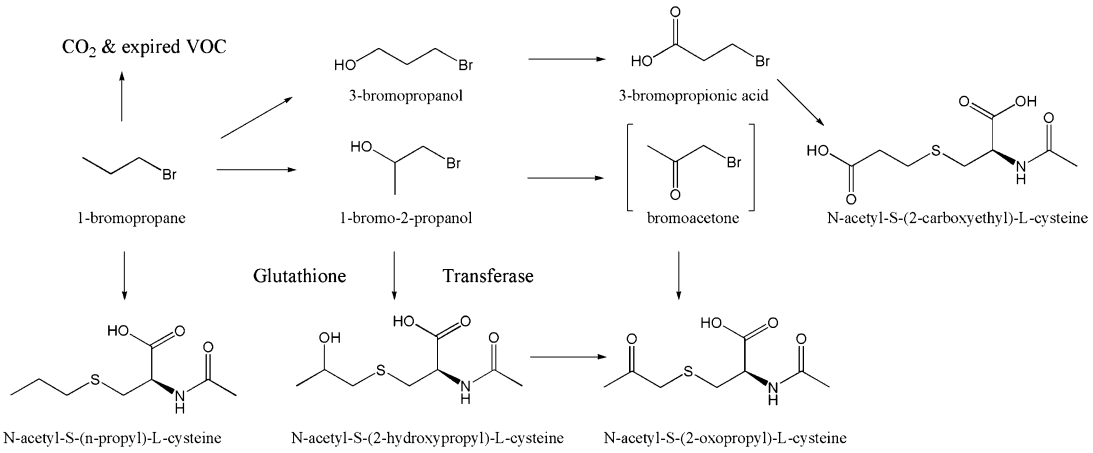

Fig. 1. Possible metabolic pathways for 1-BP.

et al., 2006). An additional urinary metabolite, AcPrCys, was analyzed from aliquots of the same urine specimens previously collected from these workers. A total of 30 workers were evaluated—13 adhesive sprayers and 17 workers performing other jobs. 'Sprayers' used 1-BP spray adhesives to assemble furniture cushions. 'Non-sprayers' included material handlers and cutters, seamstresses, and supervisors who were exposed to 1-BP vapor drifting away from spraying stations.

To evaluate 1-BP metabolites, all the workers' urine voids over a 48-h period were collected, including the amount excreted while away from work. The specimens were collected as composite samples over sequential time intervals: (i) at work, (ii) after work but before bedtime, and (iii) upon awakening. Urine collection started pre-shift before the workweek began, after nearly 3 days without exposure, and ended pre-shift 2 days later. Participants collected urine specimens in nitric acid rinsed Nalgene® bottles [high-density polyethylene (HDPE)] and immediately chilled it in small coolers with frozen gel ice. After the collection period, the total urine volume was measured with a graduated cylinder. Twenty-five milliliter aliquots were dispensed into nitric acid rinsed HDPE bottles, frozen (and shipped) on dry ice, and then stored in a laboratory freezer below −60°C.

Breathing zone (BZ) air samples were collected over these two consecutive workdays with Anasorb carbon molecular sieve sorbent tubes. The sorbent was desorbed with 1 ml of carbon disulfide and analyzed for 1-BP by gas chromatography with flame ionization detection via NIOSH method 1025 (NIOSH, 2003). The limit of detection (LOD) for this method is 1 μg which equates to a minimum detectable concentration of 0.016 p.p.m. using the maximum recommended air sampling volume of 12 l. Dermal exposure was not quantitatively measured but was qualitatively evaluated by work method observations.

The urine specimens were analyzed for creatinine (cr), $Br^{(-)}$, and AcPrCys concentrations. For comparison, single 'spot' samples were analyzed from 21 unexposed volunteers, not employed by these facilities. Creatinine was analyzed using standard spectrophotometric procedures and a Sigma Diagnostics test kit no. 555, with a LOD of 0.1 gm l^{-1}. Bromide ion was measured using an inductively coupled plasma mass spectrometer (Varion Ultra-mass 700) operated at radiofrequency of 1300 W yielding an LOD of 100 μg l^{-1}; yttrium was used as an internal standard (Allain *et al.*, 1990). Recoveries were reported to be nearly 100%, the coefficient of variation was 3%, and between-day reproducibility was ∼5%. One milliliter of each sample was diluted to 10 ml with 1% nitric acid prior to analysis. Analytical standards and quality control samples were prepared using Uri-sub, a synthetic urine solution since background concentrations of $Br^{(-)}$ may be present in pooled urine from the general population. Calibration graphs were linear over 0–40 mg l^{-1}; additional 2- or 5-fold dilutions were prepared for samples exceeding the standard calibration range. Five replicates, with 20 scans per replicate, were analyzed for each sample.

Urinary metabolites of 1-BP were characterized with a newly developed method using HPLC with ESI-MS (Cheever *et al.*, 2009). Mercapturic acid standards were prepared using the general procedure of Grenby and Young (1960) as modified by van Bladeren *et al.* (1980). Using human specimens, Cheever *et al.* (2002) isolated four mercapturic acid conjugates from workers exposed to 1-BP adhesives [e.g. AcPrCys; *N*-acetyl-*S*-(*n*-propyl)-L-cysteine-*S*-oxide; *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine; and *N*-acetyl-*S*-(3-hydroxy-*n*-propyl)-L-cysteine]. However, only the major metabolite, AcPrCys, was chosen for quantification because it was vastly predominant in worker specimens.

Urine aliquots were spiked with 10 μg ml^{-1} [d_7]-AcPrCys, an internal standard and prepared for analysis using BondElute C_{18} 500 mg solid phase extraction columns conditioned sequentially with acetone; 95% methanol:5% 0.1 N HCl and 95% H_2O (pH 3.0):5% methanol. Urine specimens were adjusted to pH 3; applied to the column; rinsed with 40% methanol:60% H_2O (pH 3.0); eluted with 4 ml acetone; and reduced to dryness under nitrogen. Eluates were dissolved in methanol and injected onto a column (150 × 2 mm, Phenomenex Aqua, 3 μm, C_{18} 300A). A 10-min linear solvent gradient was started with H_2O:methanol (85:15, 0.1% acetic acid) to 15% methanol (0.1% acetic acid) at 300 μl m^{-1}. Solvent composition was maintained with H_2O:methanol (5:95, 0.1% acetic acid). Samples were analyzed using a mass spectrometer (Agilent 1100/MSD SL ESI), and negative ions were monitored in the selectively ion mode at *m/z* 204 and 211.

Standard solutions for AcPrCys are not commercially available as an in-stock reagent, but these may be purchased from a custom synthesis laboratory. Deuterated standards and non-deuterated reference compounds were prepared by NIOSH researchers which were in excess of 98% pure as determined by HPLC-ESI/MS (Cheever *et al.*, 2009). Calibration standards were prepared at 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.625, 1.25, 5, and 10 μg ml^{-1}. Mercapturic acid conjugates are generally regarded as stable in urine (De Rooij *et al.*, 1998). AcPrCys was demonstrated to be stable with spike samples in urine extract at room temperature and chilled at 8°C for 2 weeks and repeatedly with standard solutions refrigerated at 2–4°C after numerous months of storage (Cheever *et al.*, 2009).

Control urine samples fortified with AcPrCys were similarly processed to determine recovery, LOD, and limit of quantitation (LOQ). The LOD for AcPrCys is 0.01 μg ml^{-1} and the LOQ was 0.03 μg ml^{-1}. Recovery trials were performed with blank urine fortified with 0.625, 1.25, 5, or 10 μg ml^{-1} mixed with 1 μg ml^{-1} of internal standard; spiked sample mean recovery was 96–103% ($n = 6$; SD ≤ 6.4). Calculations were based on peak area ratios of AcPrCys to [d_7]-AcPrCys. Calibration was linear from 0.01 to 10 μg ml^{-1} with correlation coefficients of 0.98 or greater and *y* intercepts that approached zero before and after each set of sample analyses. Samples out of linear range were diluted 10- or 100-fold with H_2O, pH 3, and re-analyzed.

Twenty-four and forty-eighty hour metabolite concentrations were calculated as volume-weighted concentrations for that period using at work, after work, and next day wake-up specimens. The mass was computed by summing the products of sample concentrations by the total urine volumes from each sample collected over the 24 (or 48)-h period. Linear regressions for the 48-h urinary AcPrCys concentrations versus the average 1-BP TWA concentrations for 'all jobs and both factories combined' were evaluated using both the unadjusted ($AcPrCys_{unadj}$) and creatinine-adjusted concentrations ($AcPrCys_{cr}$). Comparison of the associations showed that the relationships for creatinine-adjusted concentrations were stronger than the unadjusted concentrations ($r^2 = 0.42$ versus 0.36, respectively, for $AcPrCys_{cr}$ and $AcPrCys_{unadj}$). Hence, statistical analyses were only conducted using $AcPrCys_{cr}$ concentration and total mass.

Simple relationships for 24-h urinary AcPrCys with 1-BP TWA for each job group were also examined to assess the proportion of metabolite variability explained by 1-BP TWA. Assumptions of normality were better met by taking logarithms of the urinary AcPrCys variables. Models of urine level logarithms were regressed on the logarithm of 1-BP TWA, day, and their interaction; these were conducted separately for sprayers and non-sprayers. Terms in the model were removed when not significant. Employee was treated as a random effect, and restricted maximum likelihood was used to estimate the covariance parameters using PROC MIXED in SAS (SAS Institute, Cary, NC, USA). In addition, Spearman correlation coefficients were computed to evaluate the relationship between $AcPrCys_{cr}$ or $Br^{(-)}{}_{cr}$ concentrations with 1-BP TWA for all jobs combined and with collection intervals. Spearman coefficients were also calculated for each of the $AcPrCys_{cr}$ and $Br^{(-)}{}_{cr}$ analyses measured from the same specimens.

RESULTS

The demographic data for these workers was tabulated in the initial study (Hanley *et al.*, 2006). Most workers were female: 92.3% of the sprayers and 76.5% of the non-sprayers. Ages were also similar between job categories; average age for both groups was ∼36. Nearly half (46.2%) of the sprayers were African–American while the non-sprayers were predominately Caucasian (82.4%).

Full-shift TWA BZ concentrations to 1-BP are reported in Table 1. Adhesive sprayers' exposures were substantially greater than those for non-spraying jobs due to their immediate proximity to solvent emissions. Exposure to 1-BP among employees performing non-spraying jobs was due to ineffective general exhaust ventilation and solvent vapor drift from spraying stations. The sprayers' GM exposure to 1-BP was 92.4 p.p.m., while the non-sprayers' GM was 10.5 p.p.m. All BZ concentrations of 1-BP measured for adhesive sprayers exceeded 25 p.p.m., the industrial exposure guideline originally proposed by the EPA (2003). Eight out of 34 non-sprayers' exposures exceeded 25 p.p.m.; 23 out of 34 exceeded 10 p.p.m., the TLV® recommended by ACGIH (2009).

Table 1. Full-shift TWA BZ concentrations of 1-BP by job (p.p.m.)

Parameter	Adhesive sprayers (n = 13)	Non-spraying jobs (n = 17)
Day 1		
Minimum	44.6	0.635
Maximum	106	31.3
Average (SD)	75.6 ± 19.0	12.8 ± 9.12
GM (GSD)[a]	73.2 (1.32)	8.21 (3.30)
Day 2		
Minimum	84.8	1.63
Maximum	200	59.9
Average (SD)	121 ± 36.8	19.3 ± 15.4
GM (GSD)	117 (1.32)	13.5 (2.66)
Both days		
Average (SD)	98.4 ± 36.9	16.0 ± 12.9
GM (GSD)	92.4 (1.43)	10.5 (3.01)

SD = standard deviation.
[a]GM (GSD) = geometric mean.

Table 2. AcPrCys concentrations in urine by job for both days combined, adjusted for creatinine, mg (g-cr)$^{-1}$

Collection period[a]	Adhesive sprayers (n = 13)	Non-spraying jobs (n = 17)	Unexposed subjects[b] (n = 21)
Pre-week			
Minimum	0.682	0.0107	ND[c]
Maximum	7.41	4.85	0.318
GM (GSD)[d]	3.20 (1.90)	0.583 (5.94)	0.0235 (5.36)
Work shift			—
Minimum	9.90	0.319	—
Maximum	100	59.7	—
GM (GSD)	32.4 (1.85)	7.92 (3.36)	—
After work			
Minimum	17.5	0.422	—
Maximum	186	101	—
GM (GSD)	47.6 (1.92)	12.0 (3.41)	—
Wake up			
Minimum	15.0	0.332	—
Maximum	184	88.3	—
GM (GSD)	36.0 (1.91)	8.20 (3.70)	—

[a]Data reported by collection period for both days combined except pre-week.
[b]Unexposed subjects were office workers not employed by these facilities.
[c]ND = non-detectable. ND results were imputed for statistical calculations by dividing the LOD by the square root of 2 and adjusting for creatinine [i.e. 10 (μg l^{-1})/(square root of 2)/creatinine (gm l^{-1})].
[d]GM (GSD) = geometric mean.

In Table 2, descriptive statistics are tabulated for $AcPrCys_{cr}$ from both days combined, grouped according to collection interval and job. The sprayers' GM concentrations were ~4 times greater than those for non-sprayers. Furthermore, the GMs of pre-week $AcPrCys_{cr}$ concentrations for sprayers and non-sprayers, after an extended weekend without 1-BP exposure, were over 100 and nearly 25 times higher than that for the unexposed subjects, respectively.

Twenty-four and forty-eighty hour composite urinary AcPrCys levels were calculated to reduce the impact that individual variability of excretion rates and urination patterns may have on the time specified data (Table 3). Consistent with previous data, sprayers' GM $AcPrCys_{cr}$ concentrations were ~4 times higher than those for non-sprayers. The 48-h GM $AcPrCys_{cr}$ concentrations were 41.1 and 10.2 mg (g-cr)$^{-1}$, respectively, for sprayers and non-sprayers. Table 3 also lists the descriptive statistics for the total AcPrCys mass excreted by each worker for both days. The 48-h GM AcPrCys masses were 78.3 and 22.8 mg for sprayers and non-sprayers, respectively.

Geometric mean concentrations and 95% confidence intervals calculated from the seven sequential specimens are plotted in Figs 2 and 3, respectively, for urinary $Br^{(-)}_{cr}$ and $AcPrCys_{cr}$ concentrations. Both excretion patterns are quite similar for both sprayers and non-sprayers. For both job strata and collection interval, the $AcPrCys_{cr}$ concentrations are much lower than $Br^{(-)}_{cr}$ concentrations. The data also show an increase in excreted concentrations on the second exposure day for both Br_{cr} and $AcPrCys_{cr}$. However, interpretation of this trend is unclear since BZ exposures were also ~50% higher on the second day.

The relationship between 24-h urinary measures with 1-BP TWA are provided in Table 4 and Fig. 4 graphically shows the relation of $AcPrCys_{cr}$ with 1-BP TWA. The effect of day on either the slope or the intercept was examined for all models; neither the slope nor the intercept was modified by day for any model. For non-sprayers, there was a strong positive relationship between 24-h $AcPrCys_{cr}$ and 1-BP TWA as well as for AcPrCys mass and 1-BP TWA ($P \leq 0.0001$). Although a relationship between 24-h $AcPrCys_{cr}$ and 1-BP TWA for sprayers was observed ($P = 0.0283$), no significant relationship between 24-h AcPrCys mass and 1-BP TWA was found ($P = 0.0747$). The same general pattern was noted for $Br^{(-)}_{cr}$: the relation of $Br^{(-)}_{cr}$ with 1-BP TWA was statistically significant for non-sprayers ($P = 0.0007$) but not for sprayers ($P = 0.0536$).

Differences in urinary AcPrCys levels between the two foam plants were examined in models containing effects for plant, employee within plant, and day. The natural logarithms were used for both sprayers' and non-sprayers' urine variables. There were no significant differences between these foam cushion plants for either $AcPrCys_{cr}$ concentration or AcPrCys mass. There were also no plant differences for 1-BP TWA.

Table 3. Twenty-four and forty-eight hour urinary AcPrCys concentrations, adjusted for creatinine, and total AcPrCys mass by job

Analyte measure	Collection period	Adhesive sprayers (n = 13)	Non-spraying jobs (n = 17)
AcPrCys concentration[b], mg (g-cr)$^{-1}$	Day 1, 24-h		
	Minimum	14.3	0.373
	Maximum	97.9	37.1
	GM (GSD)[a]	32.5 (1.78)	7.77 (3.50)
	Day 2, 24-h		
	Minimum	22.1	1.23
	Maximum	127	81.5
	GM (GSD)	48.5 (1.65)	12.1 (3.04)
	Days 1–2, 48-h		
	GM (GSD)	41.1 (1.64)	10.2 (3.07)
Total AcPrCys mass[c] (mg)	Day 1, 24-h		
	Minimum	13.3	0.798
	Maximum	104	47.6
	GM (GSD)	32.4 (1.96)	8.92 (3.08)
	Day 2, 24-h		
	Minimum	15.0	1.92
	Maximum	189	66.6
	GM (GSD)	43.9 (1.91)	13.0 (2.62)
	Days 1–2, 48-h		
	GM (GSD)	78.3 (1.84)	22.8 (2.65)

[a]GM (GSD) = geometric mean.
[b]Twenty-four and forty-eight hour concentrations were calculated from composite specimens collected at work, after work, and next day wake up as volume-weighted average concentrations.
[c]Total mass was calculated by multiplying the composite concentrations by the total urine volume for each specimen followed by summation of the specimen masses for each day and 2 day period.

The relation between $AcPrCys_{cr}$ or $Br^{(-)}{}_{cr}$ with 1-BP TWA for all jobs combined and stratified by collection interval were tested using Spearman's correlation coefficients. The Spearman's coefficient data from these analyses showed that both these metabolites are highly correlated with 1-BP TWA whether partial-, 1-, or 2-day concentrations were tested [r = 0.716–0.835 for $AcPrCys_{cr}$ ($P \leq 0.0001$); r = 0.800–0.952 for $Br^{(-)}{}_{cr}$ ($P \leq 0.0001$)]. Spearman's correlation coefficients were also computed for each of the seven sequential $Br^{(-)}{}_{cr}$ and $AcPrCys_{cr}$ analyses pairs. These coefficients ranged from 0.688 for the pre-week to 0.854 for the first evening specimens; $P < 0.0001$ for each collection interval.

DISCUSSION

This study used a new HPLC tandem ESI-MS analytical method to measure several mercapturic acid conjugates possible from 1-BP metabolism. These conjugates are likely to be more specific to 1-BP than $Br^{(-)}$ since there should be less non-occupational interference. Furthermore, even though the efficacy of 1-BP analysis directly measured in the headspace of urine samples has been shown (Kawai *et al.*, 2001; Ichihara *et al.*, 2004a; B'hymer and Cheever, 2005), it is more probable to accurately measure mercapturic acid conjugates than the parent compound due to 1-BP volatility and its rapid elimination in exhaled breath (Jones and Walsh, 1979; Garner *et al.*, 2006). Headspace analysis requires immediate preparation upon collection and must be from a single sample or analyte loss could be expected. Hence, 1-BP in urine is not as practical of a biomarker for field studies, especially when monitoring many workers simultaneously both at and away from work.

Metabolism studies with propyl halides, including 1-BP, have shown that rats excreted *S*-propylcysteine, *S*-propylmercapturic acid, 2-hydroxypropylmercapturic acid, 3-hydroxypropylmercapturic acid, 2-carboxyethylmercapturic acid, propylmercapturic acid *S*-oxide, AcPrCys, and 3-bromopropionic acid in urine (Grenby and Young, 1960; Barnsley *et al.*, 1966; Baines *et al.*, 1977; Jones and Walsh, 1979). Initial analyses of human specimens in our study revealed that AcPrCys was the predominant mercapturic acid conjugate identified with this method (Cheever *et al.*, 2002). When stratified by exposure group, the same relative rankings were noted for AcPrCys as with 1-BP TWA and $Br^{(-)}$ (i.e. sprayers levels were much greater than those of non-sprayers). This method proved effective to evaluate highly exposed foam cushion workers and sensitive enough to measure lower exposed material cutters and unexposed controls.

The urinary $Br^{(-)}$ results observed for these same worker exposure days were previously reported (Hanley *et al.*, 2006). For sprayers, the 48-h GM urinary $Br^{(-)}{}_{cr}$ concentration was 195 mg (g-cr)$^{-1}$ [geometric standard deviation (GSD) = 1.23]; the non-sprayers' 48-h GM $Br^{(-)}{}_{cr}$ was 42.9 mg (g-cr)$^{-1}$ (GSD = 2.19). These levels were 40 and 10 times greater than for unexposed subjects, respectively. Strong relations of urinary $Br^{(-)}$ excretion with 1-BP exposure were found which demonstrated that urinary $Br^{(-)}$ is an important biomarker for high exposure. Moreover, the total mass of $Br^{(-)}$ from 1-BP metabolism over these 2 days were 378 mg (GSD = 1.58) and 98 mg (GSD = 1.98) for sprayers and non-sprayers, respectively. These levels in urine may implicate bromism as a plausible etiologic explanation, at least in part, to some of the observed neurologic symptoms from 1-BP exposure in reported case studies (Ichihara *et al.*, 2002; Majersik *et al.*, 2007; Raymond and Ford, 2007).

Although $AcPrCys_{cr}$ and $Br^{(-)}{}_{cr}$ results analyzed from the same specimens were highly correlated ($P < 0.0001$), some differences are apparent. First, the peak $AcPrCys_{cr}$ GM concentrations were observed

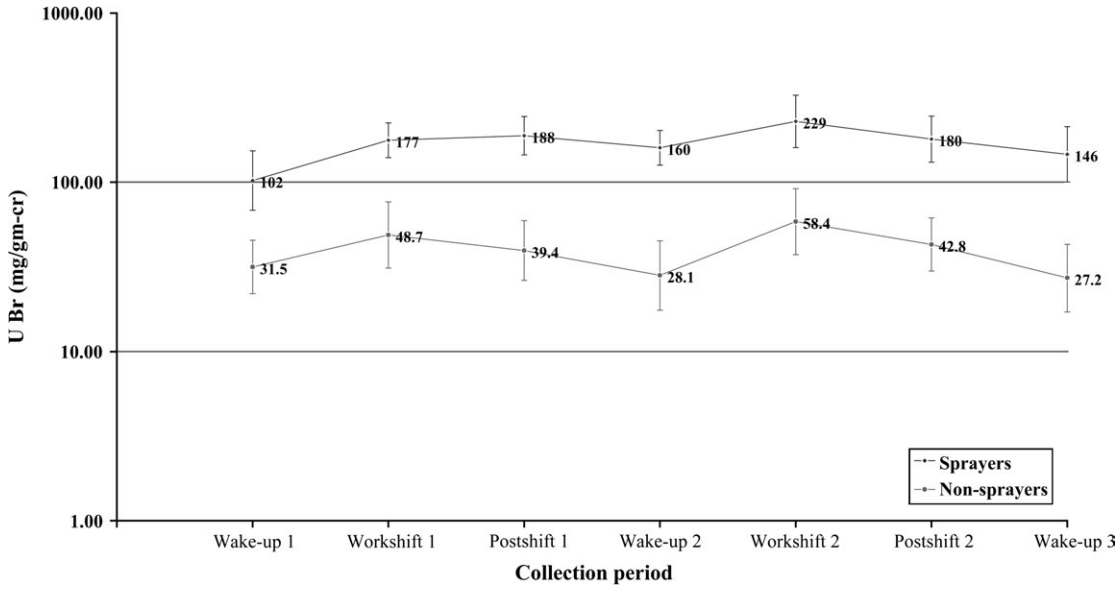

Fig. 2. Geometric mean concentrations of urinary bromide over time, adjusted for creatinine.

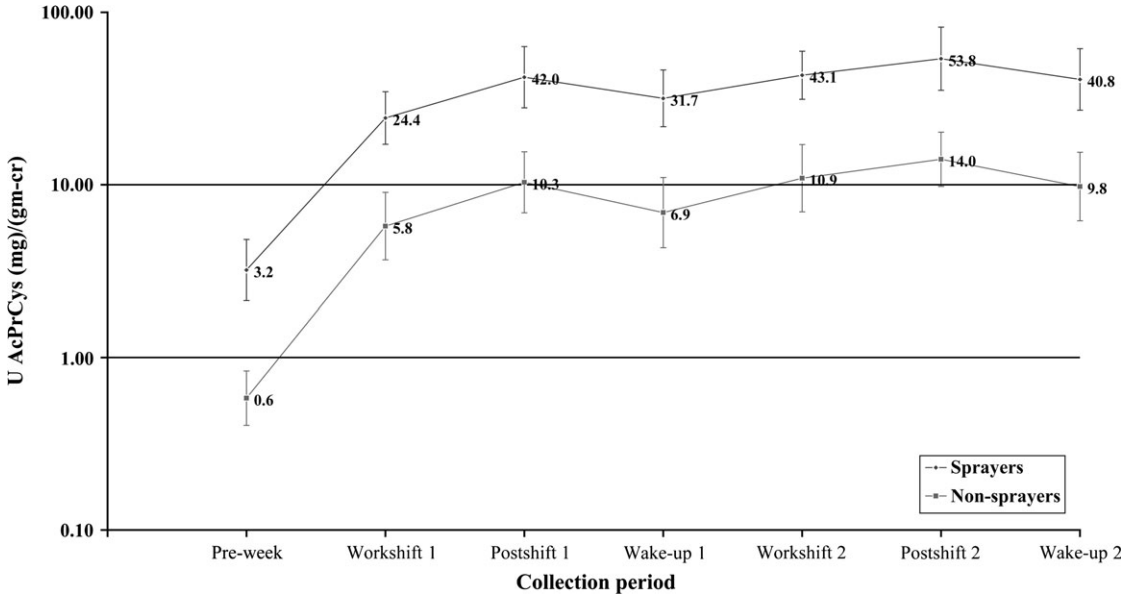

Fig. 3. Geometric mean concentrations of urinary AcPrCys over time, adjusted for creatinine.

in post-shift samples each day, whereas higher $Br^{(-)}_{cr}$ GMs were found mostly in work-shift samples (Figs 2 and 3), which suggests different elimination rates of these metabolites. In human trials, the half-life ($T_{1/2}$) of $Br^{(-)}$ in serum was reported to be 7–13 days (Vaiseman *et al.*, 1986). From Figs 2 and 3, $Br^{(-)}_{cr}$ in urine appears to have a longer ($T_{1/2}$) than $AcPrCys_{cr}$ based on the higher $Br^{(-)}_{cr}$ levels observed in pre-week specimens relative to concentrations throughout working days.

In Fig. 3, the peak $AcPrCys_{cr}$ concentrations measured post-shift decreased in the next day wake-up sample. This pattern is similar on the second exposure day except that it is slightly elevated from the day before. This figure implies that a more defined saturation plateau near the end of the workweek should occur, consistent with the rat data described by Valentine *et al.* (2007). Although pre- and post-shift specimens were not collected, all individual time interval samples were analyzed separately. Hence, the GM wake-up concentration may serve as an approximation of the next day, pre-shift concentration when adjusted for creatinine. The end-of-shift concentration most likely lies between

Table 4. Equations and *P*-values for relationships between 24-h urinary AcPrCys or bromide adjusted concentrations[a] and mass with TWA BZ concentrations of 1-BP by job[b]

Job	Parameter	24-h	
		Equation	*P*-value
Adhesive sprayers[c]	Br concentration	= −138.81 + 73.7782Ln(TWA)	0.0536
	Br mass	= 42.7951 + 34.4720Ln(TWA)	0.3889
	Ln(AcPrCys concentration)	= 1.1499 + 0.5553Ln(TWA)	0.0283
	Ln(AcPrCys mass)	= 1.4589 + 0.4761Ln(TWA)	0.0747
Non-spraying jobs[c]	Ln(Br concentration)	= 3.1309 + 0.2756Ln(TWA)	0.0007
	Ln(Br mass)	= 3.3553 + 0.2237Ln(TWA)	0.0033
	Ln(AcPrCys concentration)	= 0.4538 + 0.7731Ln(TWA)	<0.0001
	Ln(AcPrCys mass)	= 0.6363 + 0.7391Ln(TWA)	<0.0001

[a]Concentrations were adjusted for creatinine.
[b]Statistical testing was performed over both days to compute *P*-values using PROC MIXED (SAS Institute, Cary, NC, USA).
[c]For adhesive sprayers, bromide data were normally distributed and AcPrCys were log-normally distributed; both bromide and AcPrCys were log-normal for non-sprayers. Appropriate models were applied to each group.

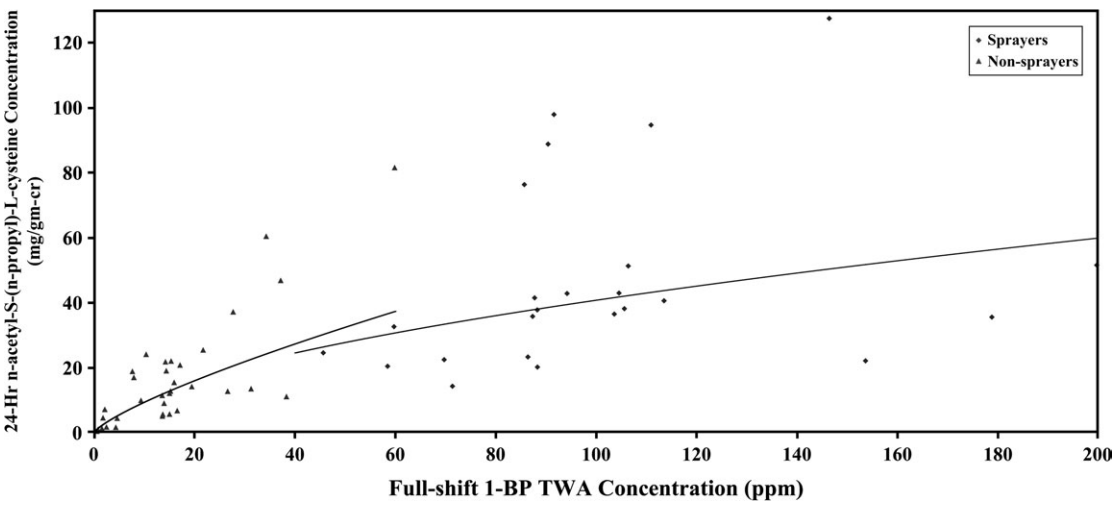

Fig. 4. Twenty-four hour urinary AcPrCys versus 1-BP TWA concentrations for workers exposed to spray adhesives, adjusted for creatinine.

the work-shift and post-shift GM concentrations since the peak GM $AcPrCys_{cr}$ concentrations were measured in the post-shift samples on each day.

The urine collection protocol of this field study does not allow for a definitive determination of AcPrCys biological $T_{1/2}$ in urine. However, these data, when considered with the literature, may allow inferences to be drawn. Valentine *et al.* (2007) evaluated the rate of elimination of AcPrCys in rats and concluded that excretion of this metabolite showed a linear dose response which appeared biphasic. Based on the data provided in that paper, the biological $T_{1/2}$ of the initial rapid, primary elimination phase was ∼18-h; the $T_{1/2}$ of the second slower phase excretion was ∼8 days. It is recognized that glutathione-*S* conjugation with halogenated hydrocarbons produces mercapturic acids that typically have relatively short biological $T_{1/2}$ (Van Welie *et al.*, 1992; De Rooij *et al.*, 1998). Two reports addressed the rates of excretion of radiolabeled 1-BP metabolites in urine of rats. Baines *et al.* (1977) found that 40–52% of the total dose was collected during the first 24-h post exposure, whereas Jones and Walsh (1979) measured 40% within 48-h. Moreover, the reduction of the GM $AcPrCys_{cr}$ concentration after work to the next day wake-up concentrations in our study (e.g. ∼24% for sprayers and 32% for non-sprayers per Fig. 3) suggests that the initial, primary $T_{1/2}$ measured in these workers is likely in the order of 1–3 days.

The GM concentration of $AcPrCys_{cr}$ from the pre-week specimens were over 100 and 20 times higher for sprayers and non-sprayers, respectively, than the GM concentration observed in unexposed subjects. These very high levels were measured following nearly 3 days without exposure. This may suggest that adipose tissue may serve as a storage depot for 1-BP similar to other low molecular weight, halogenated hydrocarbons such as vinyl chloride and

halothane (Wolff, 1976; Holaday, 1977; Gargus, 1989; Clewell *et al.*, 1995; Reitz *et al.*, 1996).

There are multiple pathways proposed for 1-BP metabolism primarily derived from animal studies; however, consensus regarding selection of a biomarker and analytical method for evaluating human exposure has not been established (Valentine *et al.*, 2007). Garner *et al.* (2006) observed that 1-BP metabolic pathways differed between species; in rats, but not mice, the predominant pathway was shown to vary with dose. The authors reported that oxidation via cytochrome P-450 produced the principal metabolite, 1-bromo-2-hydroxypropanol, which is conjugated or metabolized further. When P-450 oxidation was inhibited (or saturated at high doses), AcPrCys became predominant from S-glutathione conjugation. In the Valentine *et al.* (2007) study, a dose response of 1-BP exposure with urinary AcPrCys in rats was established and post-shift urinary AcPrCys increased with increased worker exposure to 1-BP. The authors concluded that AcPrCys could be a biomarker of human exposure to 1-BP. Our study provides additional evidence that AcPrCys, in addition to $Br^{(-)}$, can be an effective biomarker of workers' exposures to 1-BP.

Statistical comparisons of AcPrCys with the $Br^{(-)}$ data are presented in this manuscript. When jobs were stratified by sprayers and non-sprayers, statistical significance of urine metabolites with 1-BP TWA exposure, using PROC MIXED for repeated measures, was stronger for non-sprayers. The statistical significance of AcPrCys with 1-BP exposure was also greater than for $Br^{(-)}$ with 1-BP TWA. For sprayers, only $AcPrCys_{cr}$ concentrations were significant, although $Br^{(-)}{}_{cr}$ concentrations were nearly significant ($P = 0.0536$). The lower statistical relationships for sprayers may be due to frequent but variable dermal contact with wet adhesives and the slower systemic distribution and metabolism via skin absorption.

Statistical testing was conducted on 24-h metabolite concentrations as well as for collection intervals with 1-BP TWA. This required grouping normally distributed data (i.e. $Br^{(-)}{}_{cr}$ for sprayers) with log-normal data (i.e. all other data). Hence, Spearman's correlation coefficients were used since these are not affected by log transformations. In this analysis, the coefficients were slightly stronger for $Br^{(-)}{}_{cr}$ on 1-BP TWA than those for $AcPrCys_{cr}$ on 1-BP TWA and all these coefficients were highly significant. The data were also grouped according to daily collection interval to determine if a particular collection time yielded the highest correlations with 1-BP TWA exposure. However, the time interval most predictive of 1-BP TWA was not apparent, presumably due to large collection intervals (e.g. 8–10-h), variable metabolic rates and excretion patterns, and mixed exposures between consecutive days.

EPA recently published a proposed rulemaking not to accept 1-BP in aerosols and spray adhesives as an alternative to ozone-depleting solvents based on the expectation that workers would have excessive exposure to 1-BP (EPA, 2007). Indeed, the study presented here has identified many workers' exposures to grossly exceed exposure guidelines of 25 p.p.m., recommended by some solvent manufacturers and the ACGIH TLV® of 10 p.p.m. Most of the sprayers' exposures approached or were greater than an order of magnitude higher than the TLV® and many non-sprayers' exposures exceeded 10 p.p.m. This was due to spraying substantial quantities of adhesive, absence of local exhaust ventilation, and ineffective wall-mounted vane axial fans used for general ventilation. Although half-mask organic vapor respirators were available, the workers were not required to nor did they use these respirators. In addition, sprayers frequently used bare hands to pinch cushion corners and smooth edges causing substantial skin contact with adhesive. Protective gloves were not viewed as a viable solution since the glove would become glued into the cushion. Moreover, common industrial glove materials do not provide adequate protection against 1-BP permeation for extended periods. Viton® and Silvershield/4H® are reported to provide good skin protection (EnviroTech International, Inc., 2008) but these materials have not been tested with 1-BP, whereas polyvinyl alcohol and Barrier® (PE/PA/PE) materials have been shown to effectively resist 1-BP permeation for $>$8-h (Forsberg and Mansdorf, 2007).

The purpose of the biological monitoring conducted in this study was to evaluate exposure to 1-BP and excretion of metabolites. It was not intended to determine health and toxicological outcomes. Although, the health significance of these elevated metabolite levels observed at these foam cushion factories is unclear, the recently reported case studies (Majersik *et al.*, 2007; Raymond and Ford, 2007) regarding neurological toxicity in highly exposed foam cushion workers are cause for concern.

CONCLUSIONS

At these furniture cushion plants, extremely high exposures to 1-BP were measured without the benefit of effective control measures or personal protective equipment, particularly for workers using spray adhesives. Several non-sprayers' and all the sprayers' TWA BZ concentrations of 1-BP exceeded a proposed occupational exposure limit of 25 p.p.m.; most of the workers' exposures exceeded the recommended ACGIH TLV® of 10 p.p.m.

This study demonstrates that urinary elimination of AcPrCys, in addition to $Br^{(-)}$, can be an effective biomarker of 1-BP metabolism. First, when several mercapturic acid conjugates were identified from human specimens, AcPrCys was detected in the highest

quantities. Second, the effectiveness of the metabolite was demonstrated by the consistent relative ranking for all measures (e.g. 1-BP in air, urinary $Br^{(-)}$ and AcPrCys) when stratified by job exposure group. In accordance with air concentrations, urinary AcPrCys concentrations were substantially higher for sprayers than for non-sprayers. Moreover, the GM $AcPrCys_{cr}$ were two orders of magnitude greater and nearly 25 times greater for the sprayers and non-sprayers, respectively, when compared to non-exposed controls. Third, Spearman's coefficients of daily $AcPrCys_{cr}$ concentration on 1-BP TWA were highly significant. Spearman's coefficients showed urinary $Br^{(-)}{}_{cr}$ and $AcPrCys_{cr}$ were also highly correlated. More importantly, using PROC MIXED (SAS Institute, Inc.), there were strong positive, statistically significant relationships for 1- and 2-day $AcPrCys_{cr}$ and mass with BZ exposure to 1-BP in non-sprayers. For sprayers, $AcPrCys_{cr}$ concentration was associated with 1-BP, but total AcPrCys mass was not significant. Consistent with the $Br^{(-)}{}_{cr}$ findings reported by Hanley *et al.* (2006), the lower statistical association of AcPrCys and 1-BP for sprayers may be due to frequent, but variable skin contact and absorption of 1-BP.

Overall, $Br^{(-)}{}_{cr}$ concentrations and mass we previously published were substantially greater than AcPrCys levels presented here. Although urinary $Br^{(-)}$ is a useful index measured with a validated, commercially available method, AcPrCys has the advantage that it is a more sensitive analytical method with less potential interferences than $Br^{(-)}{}_{cr.}$ However, the HPLC ESI-MS methodology is more expensive requiring special equipment and knowledge.

FUNDING

Interagency agreement between the NTP; National Institute of Environmental Health Sciences (NIEHS) and the NIOSH (NIOSH Interagency Agreement Y1-ES-9045); Centers for Disease Control and Prevention (CDC).

Acknowledgements—This study was approved by and was conducted in accordance with review of the CDC-NIOSH Human Subjects Review Board (01-DSHEFS-05-XP), and the workers provided their voluntary written consent prior to their participation in the study. The authors would like to acknowledge Belinda Johnson, Brian Curwin, PhD, (NIOSH), Jason Potter, Jason Forbes, (IHI Environmental, Inc.) for field assistance; Kate Marlowe (NIOSH) for laboratory assistance; DataChem Laboratories for analytical services; Wayne Sanderson, PhD, CIH (University of Iowa) for consultation and protocol review; and Elizabeth Whelan, PhD; Cheryl Estill, MS, PE; Mark Toraason, PhD; Scott Dotson, PhD (NIOSH); Jeffrey Nemhauser, MD (CDC, NCEH) for manuscript review prior to submission to the journal. *Disclaimer*—The findings and conclusions in this report are those of the authors and do not necessarily represent the views of NIOSH. This manuscript has not been formally disseminated by NIOSH and it does not represent and should not be construed to represent any agency determination or policy. Mention of any company name or product does not constitute endorsement by NIOSH.

REFERENCES

ACGIH. (2009) 2009 TLVs® and BEIs®: threshold limit values for chemical substances and physical agents. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Allain P, Mauras Y, Douge C *et al.* (1990) Determination of iodine and bromine in plasma and urine by inductively coupled plasma spectrometry. Analyst; 115: 813–5.

Baines PJ, Bray HG, James SP. (1977) Mercapturic acid formation in the developing rat. Xenobiotica; 7: 653–63.

Barnsley EA, Grenby TH, Young L. (1966) Biochemical studies of toxic agents. The metabolism of 1- and 2-bromopropane in rats. Biochem J; 100: 282–8.

B'Hymer CB, Cheever KL. (2005) Development of a headspace gas chromatographic test for the quantification of 1- and 2-bromopropane in human urine. J Chromatogr; 814: 185–9.

Cheever KL, Marlow KL, B'Hymer C *et al.* (2009) Development of an HPLC-MS procedure for the quantification of *N*-acetyl-*S*-(*n*-propyl)-L-cysteine, the major urinary metabolite of 1-bromopropane in human urine. J Chromatogr B; 877: 827–32.

Cheever KL, Marlow KL, Stinson D *et al.* (2002) Electrospray ionization tandem mass spectrometry (ESI-MS/MS) analysis of 1-bromopropane mercapturic acid metabolites in urine (abstract). Toxicol Sci; 66: 281.

Clewell HJ, Gentry PR, Gearhart JM *et al.* (1995) Considering pharmacokinetic and mechanistic information in cancer risk assessments for environmental contaminants: examples with vinyl chloride and trichloroethylene. Chemosphere; 31: 2561–78.

Dead Sea Bromine. (1999) Applications of *n*-propyl bromide. Available at http://www.deadseabromine.com. Accessed 12 June 2009.

De Rooij BM, Commandeur JNM, Vermeulen NPE. (1998) Mercapturic acids as biomarkers of exposure to electrophilic chemicals: applications to environmental and industrial chemicals. Biomarkers; 3: 239–303.

EnviroTech International, Inc. (2006) Material safety data sheet for Ensolv™. Melrose Park, IL. Available at http://www.Ensolv.com. Accessed 28 February 2006.

EnviroTech International, Inc. (2008) Material safety data sheet for DrySolv™. Melrose Park, IL. Available at http://www.dctco.com. Accessed 31 January 2008.

EPA. (1999) Protection of stratospheric ozone: listing of substitutes for ozone depleting substances. Request for data and advanced notice of proposed rulemaking. 40 CFR Part 82. Fed Regist; 64: 8043–8.

EPA. (2000) Protection of stratospheric ozone: notice 14 for significant new alternatives policy program. 40 CFR Part 82. Fed Regist; 65: 78977–89.

EPA. (2003) Protection of stratospheric ozone: listing of substitutes for ozone depleting substances. *n*-propyl bromide. Proposed rule. 40 CFR Part 82. Fed Regist; 68: 33284–316.

EPA. (2007) Protection of stratospheric ozone: listing of substitutes for ozone depleting substances. Final rule: *n*-propyl bromide in solvent cleaning; Proposed rule: *n*-propyl bromide for use in adhesives and aerosols. 40 CFR Part 82. Fed Regist; 72: 30142–207.

Forsberg K, Mansdorf SZ. (2007) Quick selection guide to chemical protective clothing. 5th edn. Hoboken, NJ: Wiley-Interscience.

Fueta Y, Ishidao T, Ueno S *et al.* (2007) New approach to risk assessment of central neurotoxicity induced by 1-bromopropane using animal models. Neurotoxicol; 28: 270–3.

Gargus ML. (1989) Partition coefficients of low molecular weight chemicals in various liquids and tissues. Toxicol Appl Pharmocol; 98: 87–99.

Garner C, Sumner S, Davis J *et al*. (2006) Metabolism and disposition of 1-bromopropane in rats and mice following inhalation or intravenous administration. Toxicol App Pharmacol; 215: 23–36.

Great Lakes Chemicals. (2005) Material safety data sheet for *n*-propyl bromide. West Lafayette, IN: Available at http://www.e1.greatlakes.com. Accessed 7 March 2005.

Grenby TH, Young L. (1960) Biochemical studies of toxic agents: the biosynthesis of *n*-propylmercapturic acid from *n*-propyl halides. Biochem J; 75: 28–33.

Hanley K, Petersen M, Curwin B *et al*. (2006) Urinary bromide and breathing zone concentrations of 1-bromopropane from workers exposed to flexible foam spray adhesives. Ann Occup Hyg; 50: 599–607.

Holaday DA. (1977) Absorption, biotransformation, and storage of halothane. Environ Health Perspect; 21: 165–9.

Ichihara G. (2005) Neuro-reproductive toxicities of 1-bromopropane and 2-bromopropane. Int Arch Occup Environ Health; 78: 79–96.

Ichihara G, Yu X, Kitoh J *et al*. (2000a) Reproductive toxicity of 1-bromopropane, a newly introduced alternative to ozone layer depleting solvents, in male rats. Toxicol Sci; 54: 416–23.

Ichihara G, Kitoh J, Yu X *et al*. (2000b) 1-Bromopropane, an alternative to ozone layer depleting solvents, is dose-dependently neurotoxic to rats in long-term inhalation exposure. Toxicol Sci; 55: 116–23.

Ichihara G, Miller JK, Ziolkowska A *et al*. (2002) Neurological disorders in three workers exposed to 1-bromopropane. J Occup Health; 44: 1–7.

Ichihara G, Li W, Ding X *et al*. (2004a) A survey on exposure level, health status, and biomarkers in workers exposed to 1-bromopropane. Amer J Ind Med; 45: 63–75.

Ichihara G, Li W, Shibata E *et al*. (2004b) Neurological abnormalities in workers of a 1-bromopropane factory. Environ Health Perspect; 112: 1319–25.

Jones AR, Walsh DA. (1979) The oxidative metabolism of 1-bromopropane in the rat. Xenobiotica; 9: 763–72.

Jones AR, Walsh DA. (1980) The fate of *S*-propylcysteine in the rat. Xenobiotica; 10: 827–34.

Kawai T, Takeuchi A, Miyama Y *et al*. (2001) Biological monitoring of occupational exposure to 1-bromopropane by means of urinalysis for 1-bromopropane and bromide ion. Biomarkers; 6: 303–12.

Lee S, Jeon T, Kim Y *et al*. (2007) Role of glutathione conjugation in the heaptotoxicity and immunotoxicity induce by 1-bromopropane in female BALB/c mice. J Appl Toxicol; 27: 358–67.

Majersik J, Steffens J, Caravati E. (2007) Severe neurotoxicity associated with exposure to the solvent 1-bromopropane (*n*-propyl bromide). Clin Toxicol; 45: 270–6.

NIOSH. (1992) Recommendations for occupational safety and health: compendium of policy documents and statements. Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 92–100.

NIOSH. (2003)1- and 2-Bromopropane. NIOSH analytical method no. 1025. In: Eller PM and Cassinelli ME editors. NIOSH Manual of Analytical Methods. 4th edn. Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH). Publication No. 94–113. Accessed 15 March 2003.

NTP. (2004) NTP-CERHR expert panel report on the reproductive and developmental toxicity of 1-bromopropane. Center for the evaluation of risks to human reproduction. Reprod Toxicol; 18: 157–88.

OSHA. (2009) Permissible exposure limits. 29 CFR 1910.1000. Code of federal regulations. Washington, DC: U.S. Government Printing Office, Office of Federal Register.

Petroferm Inc. (2000) Technical Bulletin. Lenium® RV for asphalt extraction and binder recovery. Fernandina Beach, FL http://www.petroferm.com. Accessed July 2000.

Raymond L, Ford M. (2007) Severe illness in furniture makers using a new glue: 1-bromopropane toxicity confounded by arsenic. J Occup Environ Med; 49: 1009–19.

Reitz RH, Gargas ML, Andersen ME *et al*. (1996) Predicting cancer risk from vinyl chloride exposure with a physiologically based pharmacokinetic model. Toxicol Appl Pharmacol; 137: 253–67.

Ryan M, Baumann RJ. (1999) Use and monitoring of bromides in epilepsy treatment. Pediatric Neurol; 21: 523–8.

Takeuchi T, Okda H, Nagano K *et al*. (2001) Reproductive and developmental toxicity of 1-bromopropane in rats. Toxicol Sci; 26: 222.

Ueno S, Yoshida Y, Fueta Y *et al*. (2007) Changes in function of the inhibitory neurotransmitter system in the rat brain following subchronic inhalation exposure to 1-bromopropane. Neurotoxicol; 28: 415–20.

Vaiseman N, Koren G, Pencharz P. (1986) Pharmacokinetics of oral and intravenous bromide in normal volunteers (abstract). J Toxicol Clin Toxicol; 24: 403–13.

Valentine H, Amarnath K, Amarnath V *et al*. (2007) Globin *S*-propyl cysteine and urinary *n*-acetyl-*S*-propylcysteine as internal biomarkers of 1-bromopropane exposure. Toxicol Sci; 98: 427–35.

van Bladeren P, Buys W, Breimer D *et al*. (1980) The synthesis of mercapturic acids and their esters. Eur J Med Chem Chimica Therapeutica; 15: 495–7.

van Welie R, Van Dijck R, Vermeulen N *et al*. (1992) Mercapturic acids, protein adducts and DNA adducts as biomarkers of electrophilic chemicals. Crit Rev Toxicol; 22: 271–306.

Wang HL, Ichihara G, Ito H *et al*. (2003) Dose-dependent biochemical changes in rat central nervous system after 12-week exposure to 1-bromopropane. Neurotoxicol; 24: 199–206.

Wolff MS. (1976) Evidence for existence in human tissues of monomers for plastics and rubber manufacture. Environ Health Perspect; 17: 183–7.

Yamada T, Ichihara G, Wang H *et al*. (2003) Exposure to 1-bromopropane causes ovarian dysfunction in rats. Toxicol Sci; 71: 96–103.

Yoshida Y, Liu J, Nakano Y *et al*. (2007) 1-BP inhibits NF-kB activity and Bcl-xL expression in astocytes *in vitro* and reduces Bc1-xL expression in the brains of rats *in vivo*. Neurotoxicol; 28: 381–6.

Yu X, Ichihara G, Kitoh J *et al*. (2001) Neurotoxicity of 2-bromopropane and 1-bromopropane, alternative solvents for chlorofluorocarbons. Environ Res; 85: 48–52.